Supporting Information

of

Direct observation of time and temperature dependent transition from spherical micelles to vesicles

Hua Wei, Cui-yun Yu, Cong Chang, Chang-yun Quan, Shao-bo Mo, Si-xue Cheng, Xian-zheng Zhang* and Ren-xi Zhuo*

1. Experimental details
2. Calculation of molecular dimensions associated with the copolymer
3. Effect of acid on the transition
4. Thermoresponsive properties of vesicle(2d-20^{o}C) (LCST and size variation against temperature)
5. Possible reason leading to small size increase from micelle to vesicle determined by DLS
6. Figure S1, S2, S3, S4

1. Experimental details

Materials. *N*-isopropylacrylamide (NIPAAm) and 3-mercaptopropionic acid (MPA) were purchased from ACROS and used as received. O-(2-aminoethyl)-O'-methylpolyethylene (CH_3O-PEG-NH_2) was purchased from Fluka and used as received. 3-(trimethoxysilyl)propyl methacrylate (MPMA) was purchased from Wuhan University Chemical Plant (Wuhan, China) and used as supplied. *N*, *N*'-dimethylformamide (DMF) was obtained from Shanghai Chemical Reagent Co. and used after distillation under reduced pressure. Dichloromethane (DCM) obtained from Shanghai Chemical Reagent Co. was dried over anhydrous $MgSO_4$ and used after distillation. *N*, *N*'-azobisisobutyronitrile (AIBN) provided by Shanghai Chemical Reagent Co. was used after recrystallization with 95% ethanol. All other reagents and solvents were used without further purification.

Preparation of block copolymer based on P(NIPAAm-*co*-MPMA) and PEG (P(NIPAAm-*co*-MPMA)-*b*-PEG). P(NIPAAm-*co*-MPMA)-COOH was prepared by radical polymerization using MPA as a chain transfer agent. The molar feed ratio of NIPAAm and MPMA was determined according to our previous report.[1] NIPAAm (2.4×10^{-2} mol), MPMA (4.8×10^{-4} mol), MPA (1.77×10^{-4} mol) and AIBN (1.375×10^{-5} mol) were dissolved in 25 mL of DMF. The solution was degassed by bubbling with nitrogen for 30 min. The reaction mixture was refluxed at 70 ^{o}C for 22 h under nitrogen. Upon completion, the product was precipitated out by the addition of diethyl ether. P(NIPAAm-*co*-MPMA)-COOH was purified by repeated precipitation in diethyl ether and dried in vacuo.

P(NIPAAm-*co*-MPMA)-COOH (0.5 g), CH_3O-PEG-NH_2 (0.5 g) and *N*-hydroxysuccinimide (NHS, 2.1×10^{-4} mol) were dissolved in 8 mL of DCM. Dicyclohexylcarbodiimide (DCC, 2.1×10^{-4} mol) in 2 mL of DCM was added dropwise to the polymer solution under nitrogen atmosphere. After 24 h reaction at room temperature, the turbid mixture was filtered to remove the by-product dicyclohexylurea (DCU), then the filtrate was subjected to dialysis against 1 L of DCM using a membrane with MWCO of 8,000-12,000 Da to remove any unconjugated free CH_3O-PEG-NH_2 and

impurities. The external DCM was renewed daily for a week. The final product P(NIPAAm-*co*-MPMA)-*b*-PEG was harvested by rotary evaporating DCM followed by dried in vacuo.

Micelle-vesicle transition. In a typical procedure, P(NIPAAm-*co*-MPMA)-*b*-PEG (50 mg) was dissolved in 5 mL of DMF, which was then added quickly into 45 mL of distilled water under stirring. The temperature of the distilled water was kept constant at 60 ^{o}C. The mixture solution was kept stirring at 60 ^{o}C for 3 days, and then put into a dialysis tube and subjected to dialysis against 5 L of distilled water renewed every day for 2 weeks at 20 ^{o}C. Notice that the solutions kept stirring at 60 ^{o}C for 1 day and 2 days were performed besides 3 days in order to study the effect of thermostated time on the micelle-vesicle transition, and dialysis against distilled water at 0 ^{o}C and 40 ^{o}C were also carried out besides 20 ^{o}C to investigate the effect of dialysis temperature on the transition. Other procedures were the same as aforementioned protocols unless otherwise stated.

Measurements. The size-exclusion chromatography and multi-angle laser light scattering (SEC-MALLS) analysis were carried out to determine the molecular weights of P(NIPAAm-*co*-MPMA)-COOH, CH_3O-PEG-NH_2 and P(NIPAAm-*co*-MPMA)-*b*-PEG. A dual detector system, consisting of a MALLS device (DAWN EOS, Wyatt Technology) and an interferometric refractometer (Optilab DSP, Wyatt Technology) was used. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 0.3 ml/min. The MALLS detector was operated at a laser wavelength of 690.0 nm.

^{1}H NMR spectrum of P(NIPAAm-*co*-MPMA)-*b*-PEG was recorded on a Mercury VX-300 spectrometer at 300 MHz using $CDCl_3$ as the solvent.

TEM experiments were carried out on a JEM-2010 instrument operating at an acceleration voltage of 200 kV. TEM sample was prepared by dipping a copper grid with carbon film into the solution. After the deposition, the aqueous solution was blotted away with a strip of filter paper, stained with phosphotungstic acid aqueous solution, and then dried in air.

Optical absorbance of the vesicle aqueous solution at various temperatures was measured at 542 nm with a Lambda Bio40 UV-Vis spectrometer (Perkin-Elmer). Sample cell was thermostated in a refrigerated circulator baths at different temperatures from 22 to 46 ^{o}C prior to measurements. The LCST of the vesicle solution was defined as the temperature producing a half increase of the total increase in optical absorbance.

The average hydrodynamic diameter (D_h) at various temperatures was measured by dynamic light scattering (DLS) on a Nano Series Nano-ZS ZEN3600 (MALVERN, UK) instrument. The scattering angle was fixed at 90 ^{o}C.

2. Calculation of molecular dimensions associated with the copolymer

Two types of equations for calculating the mean square end-to-end distance ($\overline{h^2}$) of freely jointed chain under two kinds of extreme conditions were employed to estimate the range of end-to-end distance (h) for P(NIPAAm-*co*-MPMA) block, which constructs the hydrophobic membrane core of the vesicle.

In the case of extreme rigid chain:

$$\overline{h^2} = n^2 l^2 \quad (1)$$

And in the case of extreme flexible chain:

$$\overline{h^2} = nl^2 \quad (2)$$

where n is the number of bond in main chain, and l is the bond length. In this study, n = (13600/113)$\times$2 = 241 (the amount of MPMA unit is much lower than that of NIPAAm unit, therefore MPMA unit is neglected to simplify the calculation), and l = 0.154 nm (C-C bond length). On the basis of equations (1) & (2), it's found that h is 37.1 nm for extreme rigid chain, and 2.4 nm for extreme flexible chain. Then the actual value of h for P(NIPAAm-*co*-MPMA) chain should be between the two extreme values, namely, 2.4 nm < h < 37.1 nm.

In addition, the hydrophobic core thickness of resultant vesicles obtained from TEM images as shown in Fig. 1d, 2c & d, and 3a & b is around 10~20 nm, which is in the range of the theoretical calculated values based on the data of molecular weight. The results show that the dimensions of the vesicle observed by TEM are consistent with the molecular dimensions associated with the copolymer.

3. Effect of acid on the transition

As it is known, acid can catalyze the hydrolysis and cross-linking of $Si(OCH_3)_3$ groups. In order to clear the doubt whether acid will affect the formation of vesicle, a control experiment was further performed. 3 mL of 0.1 M HCl was added into the solution in the second day while stirred for 2 days at 60 ^oC, and other protocols were the same as that of vesicle (2d-20^oC). It has been found that the transition process from spherical micelle to vesicle is the same as that without addition of HCl, which reveals that acid has no obvious effect on the formation of vesicle. The reason is probably that during stirred at 60 ^oC $Si(OCH_3)_3$ groups buried in the hydrophobic PNIPAAm core could not contact with water, as a result, their hydrolysis and cross-linking could not occur. Moreover, it is reasonable postulated that even in the course of the following dialysis, the acid also has no obvious effect on the $Si(OCH_3)_3$ groups because if the cross-linking of the $Si(OCH_3)_3$ groups had occurred, the continuous transition from micelle to vesicle wouldn't have been observed as the structure and morphology is fixed after cross-linking.

4. Thermoresponsive properties of vesicle(2d-20^{o}C) (LCST and size variation against temperature)

LCST. To determine whether these cross-linked vesicles exhibit a thermal response, we examined the optical absorbance of the vesicle (2d-20^{o}C) aqueous solution as a function of temperature (Fig. S4). As given in Fig. S4, these cross-linked vesicles undergo a change in the silica cross-linked PNIPAAm

segments at a temperature corresponding to the LCST of the PNIPAAm, ca. 33 ^{o}C. Generally, in the case of a random copolymer of NIPAAm with hydrophobic comonomer, the LCST shifts to a lower temperature because incorporation of the hydrophobic comonomer facilitates chain aggregation, resulting in a decrease of the LCST.[2,3] The cross-linked vesicles, however, show the same LCST as that of pure PNIPAAm irrespective of hydrophobic MPMA units incorporation. We are led to a conclusion that the introducing of a small proportion of hydrophobic MPMA as cross-linking agent into PNIPAAm chains alters little the LCST behaviors (both response rate and LCST value). In other words, cross-linked thermoresponsive hybrid vesicles could be prepared using inorganic "silica-based" cross-linking strategy without thermoresponsivity altered. Moreover, the soluble-insoluble change is reversible as demonstrated by the transition from a turbid vesicle solution to a transparent vesicle solution when the aqueous temperature is decreased from above to below the LCST.

Size variation against temperature. We further investigated the changes of D_h of the vesicle (2d-20^{o}C) in aqueous solution as a function of temperature in the range from 25 to 48 ^{o}C. It can be seen from Fig. S4 that D_h decreases as temperature increases and D_h stabilizes at around 120 nm above 34 ^{o}C. These results are quite different from that of reported PEO-b-PNIPAAm vesicles, and in the case of PEO-b-PNIPAAm vesicles, D_h was followed while raising the solution temperature from 25 to 55 °C. Below the LCST (33 °C), the block copolymers were individual chains dispersed in solution. As the temperature approached 33 °C, aggregates with D_h > 300 nm began to form, quickly increasing in size to above 1 μm at temperatures above 40 °C.[4] However, although PNIPAAm turns to be hydrophilic below the LCST, the vesicle remains still rather than changes to unimers due to the cross-linked silica structures, and the vesicle is in a swollen state below the LCST. In addition, PNIPAAm chains shrink and turn to be more hydrophobic above the LCST, however, D_h of the cross-linked thermoresponsive hybrid vesicles reaches a platform irrespective of elevated temperature due plausibly to the stable and rigid nature-reinforced PNIPAAm wall as a result of the cross-linked inorganic silica

network, which makes it impossible for the vesicle to infinitely shrink above the LCST, instead, the shrinkage of the cross-linked vesicle is limited, thus the diameter of the vesicles keeps constant with increasing temperature. It should be also noted that the thermoresponsive PNIPAAm-*b*-PMMA micelles tend to aggregate by strengthened hydrophobic interactions at increased temperatures since the micellar outer shell (PNIPAAm segment) turns to be more hydrophobic.[5] But the thermoresponsive P(NIPAAm-*co*-MPMA)-*b*-PEG vesicles do not aggregate with increasing temperature due to the hydrophilic PEG out shells which prevent aggregation and facilitate dispersion of the vesicles.

5. Possible reason leading to small size increase from micelle to vesicle determined by DLS

The size (about 190 nm) of micelle measured by DLS (Fig. 2a) is much larger than that (70 nm on average) visualized by TEM (Fig. 2a). In our opinion, in addition to the fact that the hydrodynamic diameter determined by DLS is usually larger than the morphology size in the solid state revealed by TEM, another fact seems also to be responsible for the much larger size measured by DLS, that is, the rather white turbidity of the micelle suspension forming in the first day of dialysis probably makes it difficult for the apparatus to identify individual micelle from aggregates of micelles, and TEM observation (Fig. 2a) also reveals the dense arrangement of lots of micelles. Hence the mean size of micelles measured by DLS is likely to be the summing results of two or more micelles, which is much larger than actual value of single micelle. On the other hand, the formation of vesicle resulting from the coalescence of micelle leads to much less vesicles and their sparse arrangement, which probably makes it easier for the apparatus to determine the size of individual vesicle. Therefore, the size of vesicle (200 nm on average) observed by TEM (Fig. 2c & d) is in close accordance with that (around 260 nm) measured by DLS as shown in Fig. S3c & d. As a result, the increase of size from micelle to vesicle measured by DLS is not as evident as that observed by TEM.

References

1 H. Wei, C. Cheng, C. Chang, W. Chen, S. Cheng, X. Zhang and R. Zhuo, *Langmuir*, 2008, **24**, 4564.

2 A. Polozova and F. M.Winnik, *Langmuir*, 1999, **15**, 4222.

3 S. Koga, S. Sasaki and H. Maeda, *J. Phys. Chem. B*, 2001, **105**, 4105.

4 S. Qin, Y. Geng, D. E. Discher and S. Yang, *Adv. Mater.*, 2006, **18**, 2905.

5 H. Wei, X. Zhang, Y. Zhou, S. Cheng and R. Zhuo, *Biomaterials*, 2006, **27**, 2028.

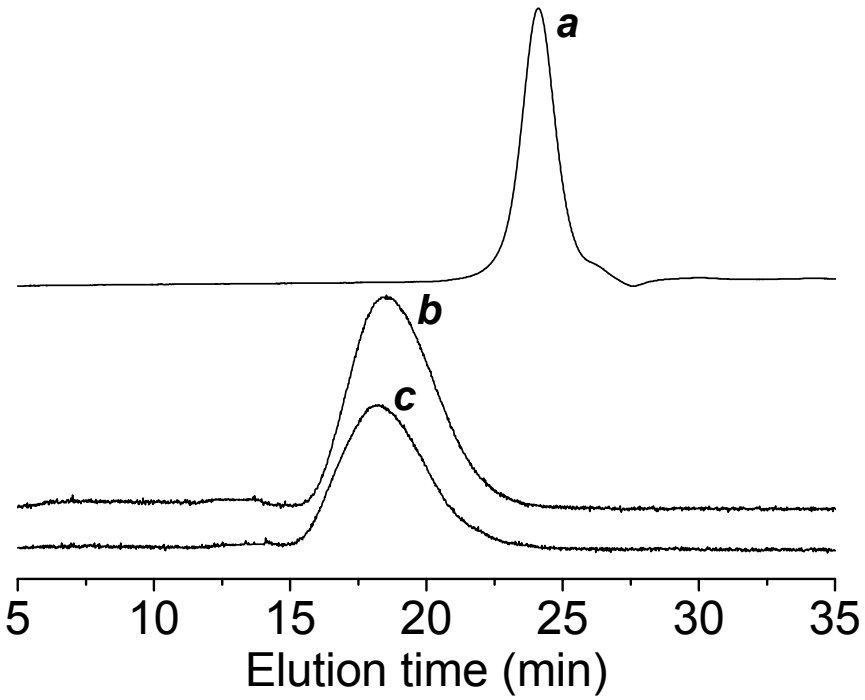

Fig. S1 SEC traces of (a) CH_3O-PEG-NH_2, M_n=2,600 Da, M_w / M_n=1.1; (b) P(NIPAAm-*co*-MPMA)-COOH, M_n=13,600 Da, M_w / M_n=1.4; (c) P(NIPAAm-*co*-MPMA)-*b*-PEG, M_n=17,500 Da , M_w / M_n=1.5.

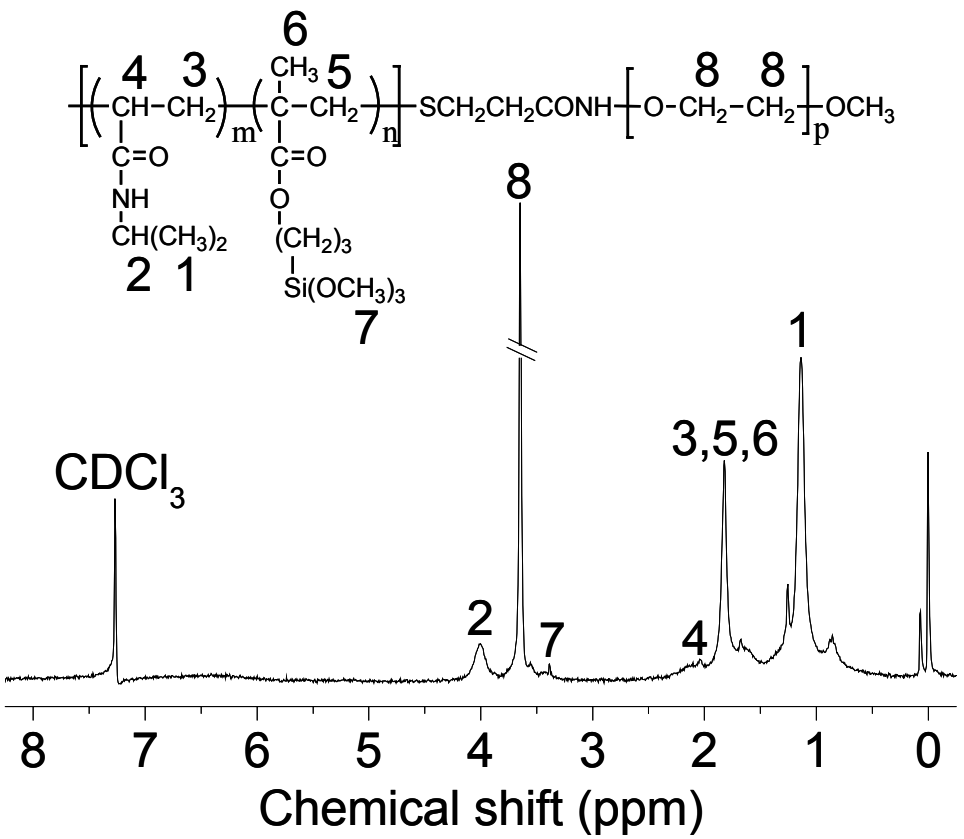

Fig. S2 ^{1}H NMR spectrum of P(NIPAAm-*co*-MPMA)-*b*-PEG copolymer in $CDCl_3$.

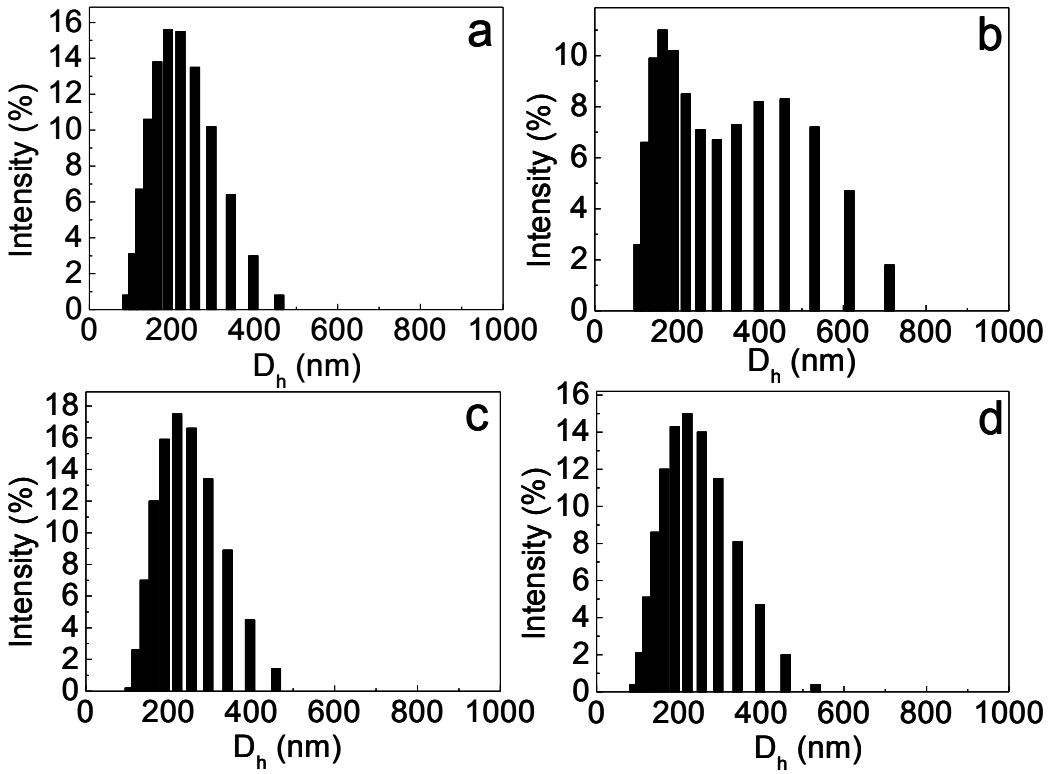

Fig. S3. Size distribution from spherical micelle to vesicle (2d-20^{o}C) in the (a) first, (b) fourth, (c) fifth day of dialysis measured by DLS and (d) size distribution of vesicle (2d-20^{o}C) after two weeks.

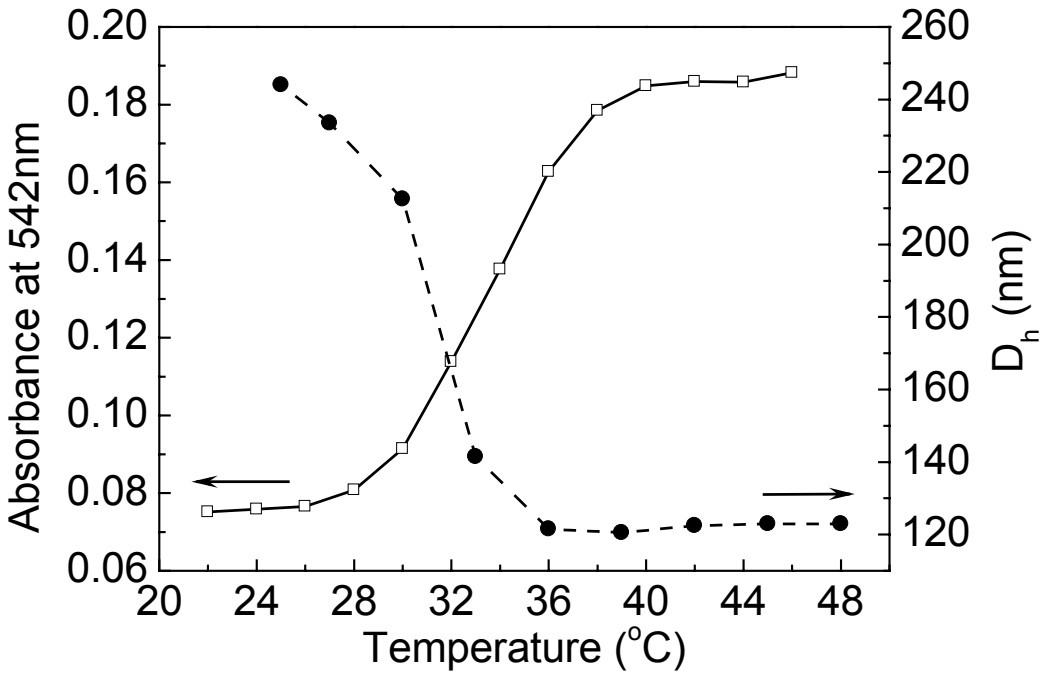

Fig. S4 Thermoresponse behaviors of Vesicle(2d-20°C) upon temperature changes. (a) LCST determined by turbidity at 542 nm, and (b) Average D_h changes as a function of temperature. Fixed angle = 90°.